Exhibit B

ASSAY FOR THE SECRETED ALKALINE PHOSPHATASE (SEAP)

1› - From the 48-hour cotransfected cell cultures, remove 250 µl of each culture supernatant and transfer into Eppendorf tubes. To be on the safe side, maintain the cultures at 37°C until satisfactory data have been obtained.

2› - Heat samples at 65°C for 5 minutes to inactivate endogenous phosphatases (Seap is stable at 65°C). homoarginine also inactivate endogenous phosphates

3› - Centrifuge for 2 minutes at room temperature in a Microfuge.

4› - Transfer 150 µl supernatants to new Eppendorf tubes. At this stage, samples may be stored at -20°C indefinately.

5› - In an Eppendorf tube, add 100 µl of 2 ~~x Seap buffer~~ to 100 µl of sample. As a Zero standard make up a mixture in triplicate substituting sample with water. Mix on a Vortex.

6› - Transfer the contents of each tube to a well of a flat bottom microtiterplate. Avoid creating air bubbles.

7› - Incubate plate at 37°C for 10 - 15 minutes.

8› - During this incubation make up the *p*-nitrophenylphosphate solution (Seap enzyme substrate) and prewarm it at 37°C for 5 minutes.

9›- Add 20 µl of the substrate solution to each well, preferably using a multipipetter.

10›- Using an ELISA microplate reader with an automatic shaker and incubator unit, measure the OD at 405 nm at regular intervals (e.g. every 5 minutes) over 60 minutes while the plate is being incubated at 37°C. (Program a 5-seconde shaking before any reading).

11›- Calculate the levels of Seap activity at a point on the curve when the changes in OD are linear with respect to time (e.g. at 15 - 30 minutes).

********** * **********

Buffer and Chemicals

2 x Seap Buffer (for 50 ml)

Amount	Stock	Final Conc.
10.51 g*	diethanolamine (100% sol.)	2 M
50 µl	1 M $MgCl_2$	1 mM
226 mg	L-homoarginine	20 mM

(*) Weigh exactly 10.51 g of diethanolamine in a tared beacker. Add distilled water up to 45 ml. Stir to homogenize. Add 50 µl of 1 M MgCl2 while stirring. In a separate 15-ml conical tube, dissolve 226 mg of L-homoarginine in 2-3 ml of distilled water. Add the solution to diethanolamine/MgCl2 solution under constant stirring. Bring up to 50 ml.

120 mM p-nitrophenylphosphate is made in 1 x Seap buffer (fresh).

Amount G (mg) = (120 mM x 371.12 x vol)/1000

Where: vol = [(# wells x 20 µl) + 100 µl extra]/1000

* Make the solution in 1 x Seap buffer (make fresh)

For 51 wells of the microplate (48 samples + 3 blanks), dissolve 50 mg *p*-nitrophenylphosphate in 1.120 ml of 1 x Seap buffer. → 600 µl stock + 600 µl H_2O

Chemicals

Name	Cat No	Storage
Diethanolamine (Fluka)	31589	Room T
L-homoarginine hydrochloride (Sigma)	H-1007	4°C
p-nitrophenylphosphate* (Fluka)	71768	4°C

(*) Also known as 4-nitrophenylphosphate disodium salt hexahydrate

0.218 / 0.226

SEAP ASSAY SHEET

I - Assay Title:

* Assay #: 72 Date #: [redacted] Investigator's Name: Wen

*Test Compounds: Mal γ & Mal.γ3Na+ transfected at [redacted] 11:30

*Concentrations:

II - DNA Transfection: Ratio (2:1) HIV/SEAP:pcTAT

*HIV/Seap: 0.4 µg/well x40 = 16.0 ug ===> From 0.376 µg/µl stock: 42.55 µl

*pcTAT: 0.2 µg/well x40 = 8.0 ug ===> From 0.639 µg/µl stock: 12.52 µl

*Total DNA (µg) = 24 ug

* Vol. Cell Fectin = Total DNA x 6 = 144 µl

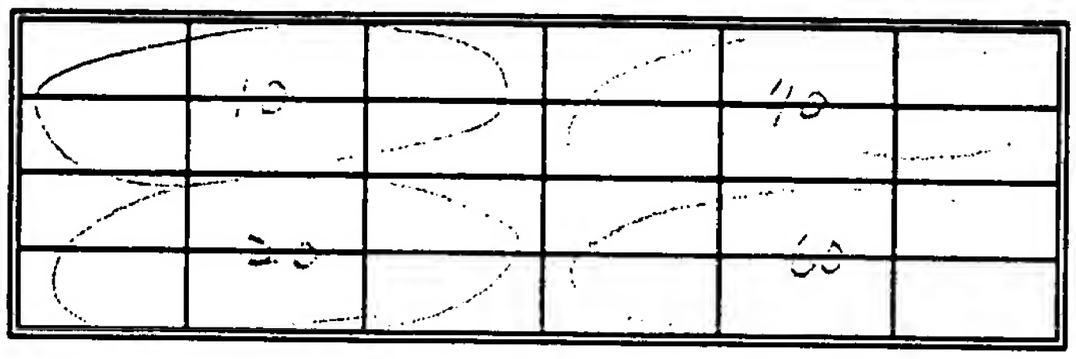
* Vol. 150 mM NaCl = Cellfectin (µl) / 0.6 = 240

* Transfection cocktail (µl)/ well: $\frac{240+240+144+42.55+12.52}{40} = 16.98$ µl

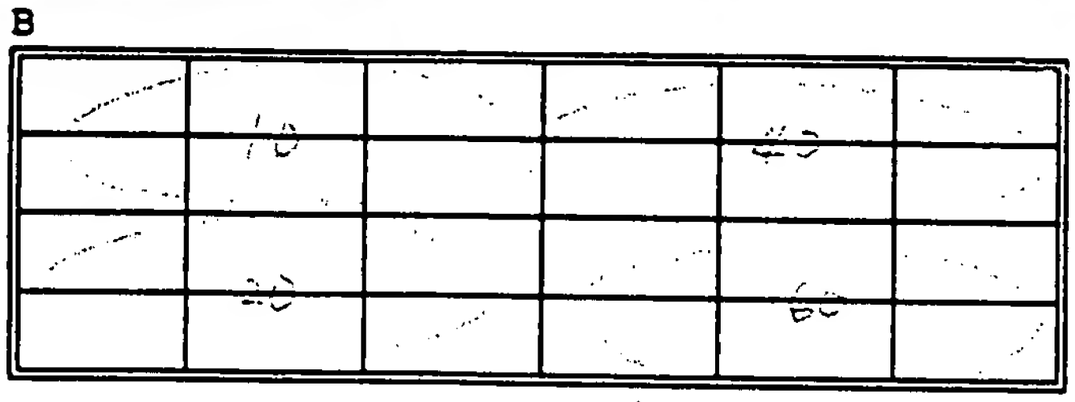
III. Linbro® 24 flat bottom well of 17 mm

Preparation of Drug conc.

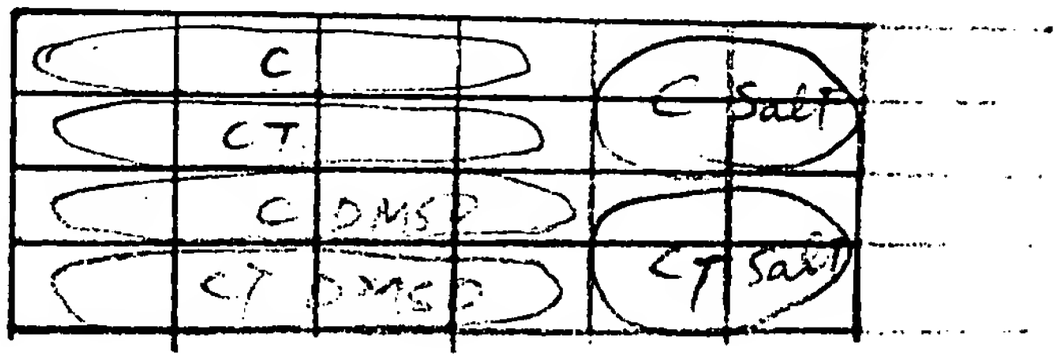
A



B



C (NOTE)



40 : 98
60 : 97

40 : 23
60 : 46

REPEATED-READS #: 0025

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TABLE OF ABSORBANCE VALUES

FILE: FILE 3 TITLE: FILE 3 REPORT

	1	2	3	4	5	6	7	8	9	10	11	12
A	0#000	-0#002	0#004	-0.217	-0.215	-0.216	0.064	0.080	0.089	0.126	0.133	0.146
							0.078			0.135		
B	-0.210	-0.214	-0.205	-0.215	-0.215	-0.213	0.151	0.153	0.207	0.124	0.153	0.155
							0.170			0.144		
C	0.070	0.016	0.060	0.044	-0.214	-0.214	0.105	0.089	0.131	~~0.362~~	0.171	0.169
			0.048				0.108				0.170	
D	0.689	0.649	0.663	0.756	-0.208	-0.210	0.126	0.122	0.120	~~0.362~~	0.172	0.197
			0.689				0.123				0.185	
E	0.094	0.100	0.081	0.110	-0.212	-0.209	0.093	0.067	0.087	0.101	0.080	0.100
			0.096				0.082			0.094		
F	0.680	0.711	0.680	0.614	-0.211	-0.213	0.830	0.776	0.832	0.649	0.587	0.501
			0.671				~~0.804~~ 0.813			0.579		
G	0.064	0.067	0.080	0.079	-0.203	-0.211	0.089	0.114	0.100	0.114	0.118	0.098
			0.073				0.101			0.110		
H	0.784	0.708	0.643	0.684	-0.209	-0.205	0.681	0.720	0.609	0.481	0.437	0.437
			0.705				0.670			0.452		

Mal.4 DMSO

C DMSO 0.641 CT

CT DMSO 0.575 E

C NaOH 0.632 G

CT NaOH H

Mal.4 - DMSO Ref: DMSO

% inhibition

10 ug/mL : 84

20 ug/mL : 97

40 ug/mL : 98

60 ug/mL : 97

Mal.4 - NaOH Ref: NaOH

% inhibition

10 ug/mL : 0 (-16)

20 ug/mL : 10

40 ug/mL : 23

60 ug/mL : 46

SEAP assay of NDGA derivatives [redacted]

1. Detach COS7 cells from four 90mm culture plates by adding 0.05% Trypsin (0.05% Trypsin in CMF-PBS, 1mM EGTA) 0.5 ml after 2 times CMF-PBS Wash.

2. Inactivate trypsin by adding 5ml complete IMDM medium (10% Fetal Bovine Serium, antibiotics) to each plate.

3. Suspend the cells by gentle pipetting. Count by Hemocytometer 160×10^4 cells/cm^3 is the cell density.

4. Seed 80ul cell suspension into 10 Linbro 24-wells culture plates which were precoated with 0.1% gelatin & contained 0.3ml complete medium each well. (1.3×10^5 cells/well) ([redacted] 18:00)

5. Transfect the cells after 26h incubation (37°C. 95% Air- 5% CO_2) by Adding 30 ul ppt (0.6 ug DNA/well) to each well that containing 0.3 ml fresh complete medium. The control wells accept same ppt without DNA. ([redacted] 20:00)

Ca-PO4 ppt preparation:

a. for control wells: Ca^{2+} soln (0.25M $CaCl_2$, 0.025M HEPES, pH 7.1) 1ml drop into 1ml bubbling PO_4^{2-} Soln (0.28M NaCl, 0.025M HEPES, ~~0.015~~ 0.0015M Na_2HPO_4, pH 7.1).

b. for transfect wells:

Three 2-ml tubes containing ~~pBC12/HIV/SEAP 18ug, pBC12/CMV/t2~~ ~~9ug,~~ 675ul PO_4^{2-} soln were added Ca^{2+} soln 675 ul ~~on~~ with DNAs (pBC12/HIV/SEAP 18ug, pBC12/CMV/t2 9ug) dropwise with bubbling. then Sit 30 min before use.

6. Incubate at 37°C for 18h.

7

Map of Plates:

I

C		
C	Mal.4	30μM
CT		
CT	Mal.4	30μM

C	#1	30μM
C	#2	30μM
CT	#1	30μM
CT	#2	30μM

II

C	#3	30μM
C	#7	30μM
CT	#3	30μM
CT	#7	30μM

C	#4	30μM
CT	#4	30μM

C	#5	30μM
CT	#5	30μM

C	#6	30μM
CT	#6	30μM

A #Mal.4

C	0μM	C	10μM	C	60μM
C	Mal.4 3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

B #1

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

C #2

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

D #3

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

E #4

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

F #5

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

G #6

C	0μM	C	10μM	C	60μM
C	30μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	30μM	CT	30μM	CT	100μM

H #7

C	0μM	C	10μM	C	60μM
C	3μM	C	30μM	C	100μM
CT	0μM	CT	10μM	CT	60μM
CT	3μM	CT	30μM	CT	100μM

C = Control wells (without DNA)
CT = Transfect wells (with DNA)

8 Remove growth medium, add 0.500ul medium containing test compounds. The final concentration of DMSO is 0.3% ([redacted]..

△ Dilution of test compounds:

Stock: MonoMe: 10.53 ug/ul use 3ul in 1ml medium = 100uM
DiMe: 11.0 ug/ul "
TriMe: 11.47 ug/ul "
tetraMe: 11.93 ug/ul "

For lower concentration, dilute the stock by DMSO. & use 3ul in 1ml medium to keep the final DMSO concentration is 0.3%.

△ DMSO stock was ~~add~~ mixed into medium just prior to use by vortexing.

9 Incubate for 48h. Remove 200ul medium from each well. for SEAP assay. ([redacted] 14:00).

10 The medium used in SEAP assay is 10ul for each sample.

MEDIMMUNE-ASSAYS #1 0625

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TABLE OF ABSORBANCE VALUES

FILE: FILE 3 TITLE: FILE 3 REPORT

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.024	0.005	0.020	-0.217	-0.216	-0.217	0.011	0.012	0.013	0.004	0.005	0.002
B	0.012	-0.008	0.012	0.012	0.020	0.008	0.002	0.004	0.005	0.003	0.019	0.003
C	-0.004	0.004	0.016	0.004	0.008	-0.001	0.585	0.704	0.631	0.558	0.394	0.421
D	1.126	1.040	1.102	0.801	0.766	0.811	0.347	0.373	0.326	0.502	0.428	0.410
E	1.010	0.855	0.915	0.890	0.753	0.799	0.018	0.020	0.007	0.012	-0.006	0.023
F	-0.213	-0.216	-0.215	-0.213	-0.213	-0.216	0.008	0.014	0.007	-0.010	0.010	0.036
G	0.007	0.011	0.013	0.005	0.001	0.017	1.323	1.433	1.105	1.111	0.292	0.269
H	0.007	-0.001	0.016	0.003	0.009	0.026	1.284	1.234	0.783	0.715	0.165	0.162

30uM inhibition

Mol.%	% inhibition
14.9	=
28.0	=
25.3	=
42.1	=
51.5	=
62.8	=
61.7	=
68.2	=

Mol.%	% inhibition
3uM	= 8.2
10uM	= 19.2
30uM	= 44.7
60uM	= 80.0
100uM	= 89.6

[redacted]-READS # 3625

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TABLE OF ABSORBANCE VALUES

FILE: FILE 3 TITLE: FILE 3 REPORT

	1	2	3	4	5	6	7	8	9	10	11	12
A	0#002	-0#004	0#003	-0.211	-0.209	-0.212	0.008	0.016	0.003	-0.001	0.002	0.016
B	1.239	1.354	1.209	1.239	0.420	0.350	1.122	1.205	0.921	0.933	0.208	0.218
C	-0.005	0.007	0.008	0.004	0.007	-0.001	1.200	1.149	0.871	1.088	0.374	0.357
D	1.365	1.306	1.025	1.098	0.423	0.464	1.067	1.185	0.570	0.530	0.236	0.226
E	1.288	1.327	0.905	1.005	0.330	0.314	0.017	0.012	0.002	0.007	0.003	0.016
F	-0.202	-0.210	-0.210	-0.210	-0.209	-0.209	0.006	0.010	0.009	0.002	0.004	0.010
G	1.239	1.354	1.209	1.239	0.420	0.350	1.122	1.205	0.921	0.933	0.208	0.218
H	1.387	1.279	0.682	0.580	0.203	0.193	1.178	1.165	0.475	0.435	0.085	0.114

B

#1

C

#2

D

#3

E

#4

#1
% inhibition

3 uM : -3.3 (0)
10 " : 5.7
30 " : 52.8
60 " : 70.8
" : 86.0

#2
% inhibition

3 uM : 2.8
10 " : 21.9
30 " : 29.4
60 " : 67.8
100 " : 76.3

#3
% inhibition

3 uM : 3.7
100 uM : 15.8
30 uM : 52.5
60 uM : 69.3
100 uM : ~~80~~ 81.0

#4 % inhibition

3 uM : -1.3 (0)
10 uM : 19.8
30 uM : 60.9
60 uM : 82.4
100 uM : 92.2

REPEATED-READS #: 0025

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TABLE OF ABSORBANCE VALUES

FILE: FILE 3 TITLE: FILE 3 REPORT

	1	2	3	4	5	6	7	8	9	10	11	12
A	0#000	0#008	0#010	0.210	0.208	0.210	0.209	0.209	0.210	0.208	0.209	0.209
B	0.207	0.208	0.209	0.208	0.208	0.209	0.208	0.209	0.209	0.208	0.209	0.211
C	0.012	0.014	0.012	0.022	0.017	0.020	0.011	0.014	0.011	0.014	0.019	0.015
D	0.007	0.013	0.015	0.014	0.026	0.012	0.010	0.019	0.004	0.020	0.038	0.043
E	1.192	1.134	0.699	0.687	0.277	0.262	1.391	1.489	0.658	0.623	0.317	0.300
F	1.215	1.138	0.374	0.400	0.202	0.184	1.163	0.974	0.380	0.317	0.189	0.239
G	0.205	0.208	0.208	0.206	0.199	0.208	0.206	0.207	0.207	0.207	0.209	0.210
H	0.207	0.207	0.204	0.206	0.206	0.204	0.189	0.207	0.207	0.206	0.206	0.210

G #6: C 1&2 = 0.013; C 3&4 = 0.017; C 5&6 = 0.019; D 1&2 = 0.010; D 3&4 = 0.015; D 5&6 = 0.019; E 1&2 = 1.163; E 3&4 = 0.693; E 5&6 = 0.270; F 1&2 = 1.177; F 3&4 = 0.387; F 5&6 = 0.193

H #7: C 7&8 = 0.013; C 9&10 = 0.013; C 11&12 = 0.017; D 7&8 = 0.015; D 9&10 = 0.012; D 11&12 = 0.041; E 7&8 = 1.440; E 9&10 = 0.641; E 11&12 = 0.309; F 7&8 = 1.069; F 9&10 = 0.349; F 11&12 = 0.214

#6
% inhibition

3 uM : -1.5 (0)
10 uM : 41.0
30 uM : 67.7
60 uM : 78.2
100 uM : 84.9

#7
% inhibition

3 uM : 26.1
10 uM : 56.0
30 uM : 76.4
60 uM : 79.5
100 uM : 87.9

REPEATED-READS #: 0025

[redacted] 10:33:14.31 PM

TABLE OF ABSORBANCE VALUES

FILE: FILE 3 TITLE: FILE 3 REPORT

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0#005	-0#012	0#018	-0.209	-0.210	-0.211	-0.210	-0.209	-0.208	-0.209	-0.210	-0.211
B	-0.208	-0.209	-0.209	-0.209	-0.209	-0.209	-0.209	-0.209	-0.209	-0.209	-0.207	-0.211
C	0.028	0.031	0.037	0.037	0.040	0.041	-0.206	-0.207	-0.209	-0.208	-0.208	-0.209
D	0.015	0.028	0.036	0.028	0.047	0.035	-0.208	-0.210	-0.208	-0.208	-0.208	-0.209
E	1.500	1.394	0.701	0.680	0.318	0.291	-0.207	-0.207	-0.208	-0.207	-0.209	-0.209
F	1.131	1.033	0.385	0.417	0.222	0.185	-0.207	-0.208	-0.207	-0.206	-0.208	-0.213
G	-0.206	-0.206	-0.208	-0.207	-0.206	-0.208	-0.206	-0.205	-0.207	-0.207	-0.208	-0.208
H	-0.207	-0.204	-0.209	-0.208	-0.207	-0.207	-0.207	-0.207	-0.207	-0.207	-0.207	-0.207

#5 B

C: 0.030 0.037 0.041

D: 0.022 0.032 0.041

E: 1.447 0.691 0.305

F: 1.082 0.401 0.201

#5 % inhibition

3 uM : 25.2

10 uM : ~~53.8~~ 53.8

30 uM : 74.0

60 uM : 81.4

100 uM : 88.5

% Inhibition of NDGA Derivatives on SEAP assay.

	Mal.4	#1	#2	#3	#4	#5	#6	#7
3 μM	8.2	0 (-3.3)	2.8	3.7	0 -1.3	25.2	0 -1.5	26.1
10 μM	19.2	5.7	21.9	15.8	19.8	53.8	40.1	56.0
30 μM	44.7	~~52.8~~	29.4	52.5	60.9	74.0	67.7	76.4
60 μM	80.0	~~70.8~~	67.8	69.3	82.4	81.4	78.2	79.5
100 μM	89.6	86.0	76.3	81.0	92.2	88.5	84.9	87.9

Figure B(1)

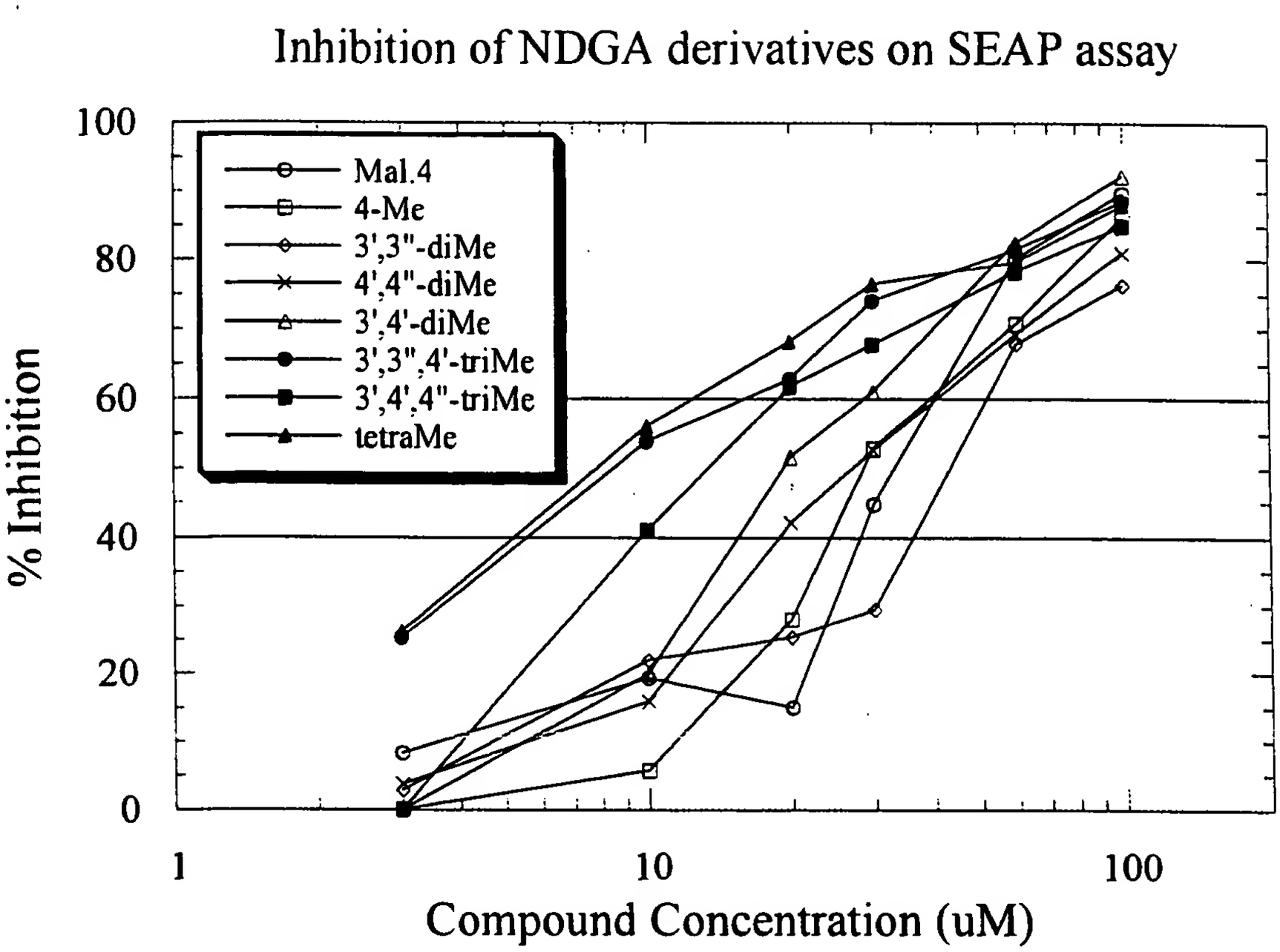

Figure B(2)